Example 5: Cancer Bearing Mice Life Prolongation Test

A. Method

[0053] Fifty (50) C57BL/6J mice weighing 18-22 g, without sex discrimination, were randomly assigned to one control group and four treatment groups, each with 10 animals. Murine sarcoma 180 cells were intraperitoneally transplanted to the mice. 20(S)-Rh2 was provided by Shenyang Pegasus Pharmaceutical R&D Co., China, with a purity of over 98%. The molecular weight for Rh2 was 622.3. The novel sapogenin PAM-120 was derived from the process described in Example 2 according to this invention. The molecular weight of PAM-120 was 442.7, and the purity for PAM-120 was higher than 99%. The drugs were prepared into a suspension form respectively. The mice were weighed daily prior to drug administration to determine the actual measurement of drug administered. Drug administration started from 24 hours post tumor inoculation. The mice in the two low-dose treatment groups were orally given the Rh2 and PAM-120 preparations using a gastric catheter at a daily dose of 10 mg/kg of Rh2 and 10 mg/kg of PAM-120 respectively for a lifetime or up to 120 days. The mice in the two high-dose treatment groups were orally given the Rh2 and PAM-120 preparations using a gastric catheter at a daily dose of 25 mg/kg of Rh2 and 25 mg/kg of PAM-120 respectively for a lifetime or up to 120 days. The mice in the control group were orally given a normal saline. For each group, the days of survival for 50% animals (DS_{50}) and the average days of survival (ADS) were recorded. For groups containing one or more animals that could have lived longer than 120 days (the designed sacrifice day was d 120), the ADS would be so calculated that these animals were counted as if they had died on day 120, and a note would be made. The life prolongation rate (LPR) was calculated with the following formula:

$$LPR(\%) = \frac{ADS_{(treatment)} - ADS_{(control)}}{ADS_{(control)}} \times 100\%$$

B. Result

Table 4. B16 MELANOMA BEARING MICE
LIFE PROLONGATION RATE

Group	DS_{50}	ADS(M∓SD) %	LPR(%)
Control	14	14.7 ∓ 5.4	
Rh2 (3 mg/kg)	22	24.7 ∓ 12.6	68.0
PAM-120 (3 mg/kg)	38	38.6 ∓ 16.4	162.6
Rh2 (6 mg/kg)	41	44.3 ∓ 19.6	201.4
PAM-120 (6 mg/kg)	77	80.6 ∓ 34.4	448.3

[0054] The anti-cancer effect of the novel sapogenin PAM-120 was indicated by the significant increase in life prolongation of the mice bearing murine sarcoma ($P<0.01$ compared with the average days of survival in the control). Better anti-cancer effect of novel sapogenin PAM-120 on murine sarcoma than that of Rh2 was demonstraated by the significant increase in life prolongation of the sarcoma-bearing mice ($P<0.01$, compared with the average days of survival in the relevant Rh2 treatment dose groups). Two mice in the 25 mg/kg composition treatment group survived for the whole 120 days, and were found to have no tumors whatsoever existing in their bodies postmortally.

[0055] Figure 1 illustrates a graph of tumor inhibiting effect of various ginsenosides on B16 cells. Mouse melanoma tumor B16 cells were cultured with DMEM and 5% serum supplement in 96-well dishes. Cells were then treated with various concentrations of PAN-20, PAN-30, PBM-100, PBM-110, PAM-120 and Rh2, respectively. The number of alive cells were measured using MTT method 24 hours after the treatment and compared with the control samples. All the new compounds showed a significantly higher tumor inhibitory effect than RH2, especially at low concentrations ($p<0.01$, Student t test).

[0056] Figure 2 illustrates a graph of tumor inhibiting effect of various ginsenosides on drug resistant human breast cancer cells MCF7r. Human drug resistant breast cancer cells (MCF7r) were cultured with DMEM and 5% serum supplement in 96-well dishes. Cells were then treated with various concentrations of PAN-20, PAN-10, PAN-12 and Hr2, respectively. The number of alive cells

were measured using MTT method 24 hours after the treatment and compared with the control samples. All new compounds showed a significantly higher tumor inhibitory effect than Rh2, especially at low concentrations ($p < 0.01$, Student t test).

[0057] Figure 3 illustrates a plot of the synergistic effect of PAM-120 with Cisplatin on drug resistant human breast cancer cells MCF7r. MCF7r cells were treated with anti-cancer chemotherapy agent Cisplatin at various concentrations in the presence of 10 ug/ml PAM-120. In Figure 3, the first bars in each concentration group represent percentages of alive cells 24 hours after treatment with Cisplatin only. The second bars in each group represent the results of cells treated with Cisplatin and PAM-120.

[0058] Figure 4 illustrates a plot of the synergistic effect of PAM-120 with Taxol on drug resistant human breast cancer cells MCF7r. MCF7r cells were treated with anti-cancer chemotherapy agent Taxol at various concentrations in the presence of 10 ug/ml PAM-120 or 20 ug/ml RH2. In Figure 4, the first bars in each concentration group represent percentages of alive cells 24 hours after treatment with Taxol only. The second bars in each group represent the results of cells treated with Taxol and PAM-120, while the third bars represent the results of cells treated with 20 ug/ml Rh2.

[0059] Figure 5 illustrates a graph of the therapeutic effect of PAM-120 on mouse intracranial human malignant glioma (U87) model. Nude mice were intracranially implanted with human malignant glioma cells (U87). On day 10 post tumor implantation, animals were treated with various dosages of PAM-120. Animals treated with 25 mg/kg and 50 mg/kg PAM-120 had significantly longer survival time after tumor implantation ($p < 0.01$, Kaplan Meier analysis).

[0060] Figure 6 illustrates a graph of the therapeutic effect of PAM-120 on mouse subcutaneous human malignant glioma (U87) model. Nude mice were subcutaneously implanted with human malignant glioma cells (U87). On day 7 post tumor implantation, animals were treated with 25 mg/ml PAM-120 or equal dose of Rh2. Tumor sizes were measured on day 7 (before treatment) and day 24 (after the treatment). Both PAM-120 and Rh2 significantly inhibited tumor growth comparing to PBS control animals. Tumor sizes in PAM-120 treated